Supplementary information

Cover sheet

Title	**Response of algal-bacterial regrowth characteristics to the hypochlorite in eutrophic landscape ponds replenished with reclaimed water**
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The number of papers	8
The number of figures	2
The number of tables	3

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Figure captions

Fig. S1 Method evaluation for spectrophotometric determination of algal density.
Fig. S2 The effect of sodium hypochlorite on bacterial regrowth in reclaimed water in the dark experiment.
Fig. S3 The interaction network of bacteria community at the class level under (a) chlorination and (b) non-chlorination conditions.

Table Captions

Table S1 The water quality of RW and LW
Table S2 The dominant symbiotic bacteria taxa reported in previous studies
Table S3 The lifestyle of the potentially pathogenic bacteria in this study

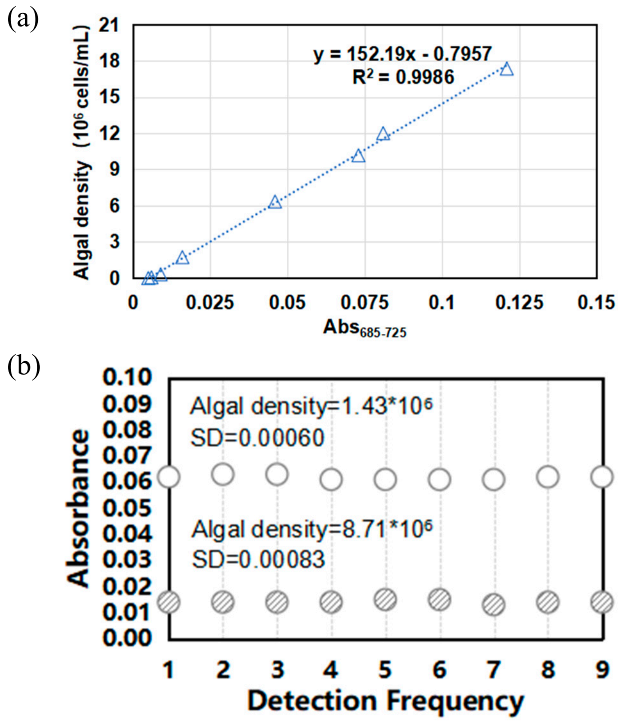


Figure S1. Method evaluation for spectrophotometric determination of algal density. (a) the standard curve between the algal density and the absorbance. (b) The method detection limit (MDL), calculated based on the EPA's procedure, is equivalent to three times the standard deviation of replicate instrumental measurements of spiked blanks. Nine replicates were performed (a measurement with three replicates, a re-zero of the instrument and three replicate measurements, a restart of the instrument and three replicate measurements). Therefore, the MDL was $0.11*10^6$ cells/mL [=152.19*1/2(0.00060+0.00083)]. The sensitivity (S) of the method is proportional to the sensitivity of the instrument. Therefore, the S was $0.15*10^6$ cells/mL [=152.19*0.001].

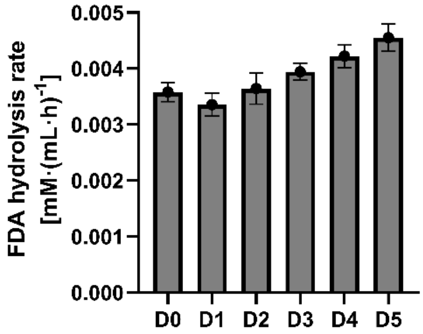


Figure S2. The effect of sodium hypochlorite on bacterial regrowth in reclaimed water in the dark experiment. the group "D0" means no chlorine addition, and "D1" ~ "D5" were the same meaning as in the main text. The differences in the number of bacteria were not as dramatic as those in the light/dark cycle experiment.

Test design:

The additional test was designed to assist in answering the contribution of algal growth to the increase of bacterial abundance.

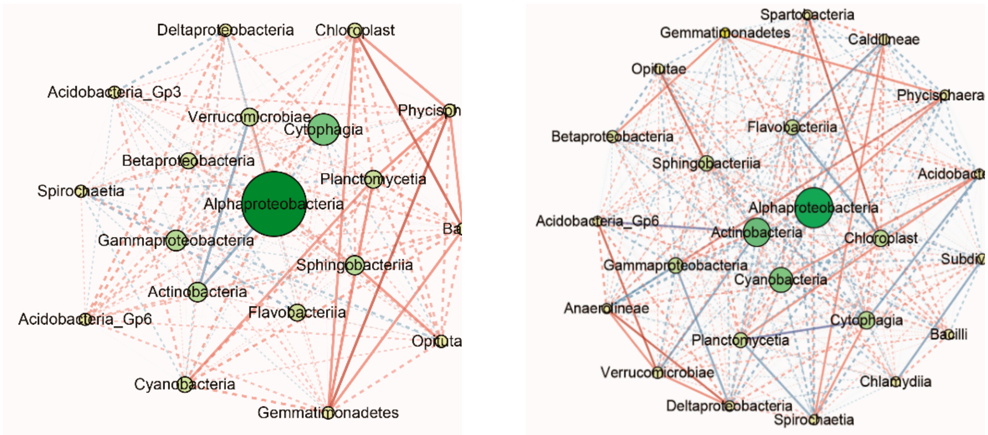
Basic experimental procedures were the same as those described in the text. The only difference was that the mixtures were placed in a constant-temperature incubator without illumination. Accordingly, algae regrowth was significantly inhibited. The results illustrated the characteristics of bacterial regrowth potential without symbiotic algae growth. Hence, a comparison can be made with the microcosm experiment in the main text to provide a preliminary explanation.

The nutrients' conditions:

Indicators	Values	Unit
TN	ca. 10	mg/L
Nitrate nitrogen	ca. 9	mg/L
TP	ca. 0.3	mg/L
Soluble reactive phosphorous (SRP)	ca. 0.26	mg/L
pH	7.8~8.2	
TDS	950	mg/L
ORP	180~205	mV

The indicator and determination method:

Samples were collected every day to monitor the OD600 to briefly grasp the dynamic of the bacterial population. The total bacteria population was characterized by the total bacterial activity using the fluorescein diacetate hydrolysis (FDA) method [1]. It was determined when the OD600 of samples reached the maximum (i.e., the stationary phase).



Number of nodes	**19**	**Number of nodes**	**24**
Number of edges	**171**	**Number of edges**	**276**
Avg. Number of Neighbors	**18.0**	**Avg. Number of Neighbors**	**23.0**

Figure S3 The interaction network of bacteria community at the class level under (a) chlorination and (b) non-chlorination conditions. The size of nodes represents the average relative abundance among the groups D1~D5. The lines represent the positive (red) or negative (blue) correlation between the two classes, and line width means the correlation degree. Lines with solid type represent the correlation with a p-value < 0.05.

Table S1 The water quality of RW and LW

Index	LW[a]	RW	DB12 599-2015
TN (mg/L)	1.99~10.25	8.77~10.3	10
NO_3^--N (mg/L)	1.02~3.40	6.93~8.81	--
NH_4^+-N (mg/L)	0.99~1.51	0.22~1.31	1.5(3.0[c])
TP (mg/L)	0.20~0.29	0.25~0.32	0.3
SRP[b] (mg/L)	0.02~0.04	0.20~0.28	--
CODcr (mg/L)	39~43	20~24	30
pH	8.7~9.2	7.8~8.2	6~9
ORP (mV)	208~247	220~250	--
TDS (mg/L)	2550~4780	1840~2660	--
Turbidity (NTU)	24.1~35.3	2.1~3.6	--
Chla (μg/L)	0~24.2	Not detectable	--

a. The upper limit values of the above range occurred in the area of replenishment of RW. Lower limit values generally occurred in areas far from the outfall.
b. SRP is the abbreviation of soluble reactive phosphorus.
c. The value in the parentheses represent the limit to be enforced in winter.

Table S2 The dominant bacteria taxa symbiotic with algae that reported in previous studies

Dominant taxa	Symbiotic system	Reference
B. Flavobacteriales_Flavobacteriaceae A. Rhodobacterales_Roseobacter clade B. Flavobacteriales_Muricauda G. Pseudomonadales_Marinobacter	with *Tetraselmis suecica P039*	[2]
G. Moraxellales_Acinetobacter, G. Unclassified_Pseudomonadales Bacteroidetes	Algal-bacterial consortia	[3]
B. Flavobacteriia_Flavobacteriales G. Alteromonadales	with Alexandrium fundyense; Dinophysis acuminata	[4]
G. Vibrionales _*Vibrio spp.* A. Rhodobacterales_*Sediminimonas* Other *Alphaproteobacteria*	with Akashiwo sanguinea	[5]
B. Flavobacteriales_Flavobacterium B. Sphingobacteriales B. Cytophagales_Cytophagaceae A. Rickettsiales	Algal-bacterial consortia	[6]
A. Caulobacterales_Phenylobacterium, A. Caulobacterales_Brevundimonas, A. Hyphomicrobiales_Phyllobacterium, A. Hyphomicrobiales_Afipia, A. Sphingomonadales_Sphingomonas, A. Sphingomonadales_Sandaracinobacter, G. Cellvibrionales_Cellvibrio	with Chlorella vulgaris	[7]
B. Cytophagales B. Flavobacteriales A. Rhizobiales	with Nannochloropsis	[8]
A. Rhidobacterales_Rhodobacteraceae Lactobacillales_Trichococcus G. Xanthomonadales_Aquimonas G. Chromatiales_Chromatiaceae F. Caldilinea_Caldilinea B. Flavobacteriales_Flavobacterium	High-Rate Algal Pond	[9]
B. Flavobacteriales_Polaribacter spp. B. Flavobacteriales_Flavobacteriaceae B. Flavobacteriales_Formosa spp. B. Flavobacteriales_Prosiliicoccus spp. B. Flavobacteriales_Aurantivirga spp. NS3a marine group NS5 marine group	Algal bloom in surface seawater at Helgo- land Island	[10]
B. Flavobacteriales_Ulvibacter B. Flavobacteriales_Prosiliicoccus	Algal bloom in surface seawater at Helgo- land Island	[11]

Notes: the prefix of "A." in taxa names means the Class *Alphaproteobacteria*, "B." means Phylum *Bacteroidetes*, "G." means the Class *Gammaproteobacteria*, and "F." means the Phylum *Firmicutes*.

Table S3 The lifestyle of the potentially pathogenic bacteria in this study

Genus	life state	Gram Positive/Negtive	Traits	Reference
Pseudomonas	Mainly Free-floating (FL) /Little Particle-Attached (PA)	Gram-negative	Animal/plant pathogen Plant Growth Promoting Rhizobacteria	[12]
Rhizobium	FL	Gram negative	Phycosphere	[13,14]
Mycobacterium	PA	Neither Gram-Positive OR Gram-negative	Animal pathogen/Phycosphere	[12]
Orientia	Unknown	Gram-negative	Unclear, probably phycosphere	--
Sphingomonas	PA	Gram-negative	Phycosphere	[12,15]
Legionella	FL/Little PA	Gram-negative	Opportunistic pathogen, causing legionnaires disease	[12]
Bacillus	FL	Gram-positive	Plant Growth-Promoting	[16,17]
Leptospira	FL	Gram-negative	Zoonotic pathogen Causing acute flulike febrile illnesses	[12]
Streptococcus	PA	Gram positive	Fish/Shellfish pathogen	--
Pseudonocardia	--	Gram-positive	--	--

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